Mating Type Distribution, Fungicide Sensitivity, and Phylogenetic Relationships of *Phytophthora colocasiae*

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ABSTRACT

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From 1997 to 2011, ***Phytophthora colocasiae*** was isolated from taro leaf blight samples, collected from the islands of Oahu, Kauai, Molokai, and the Big Island of Hawaii. A total of 218 isolates were obtained and all were of the A2 mating type. Isolates collected in 2011 from 20 farms in 12 districts of the 4 islands were also tested for metalaxyl resistance. No resistant isolates were found from Kauai, Molokai or the Big Island of Hawaii. However, 25 percent of the isolates from Oahu were resistant to metalaxyl. Radial growth of 112 isolates tested ranged from 13 to 17 mm on V8 agar after 4 days at 24 ˚C. Two types of colony morphology were observed: one with sparse aerial mycelia, and the other with dense, cottony aerial mycelia. No chlamydospores were observed in either type. Eighteen of 21 isolates from Hawaii clustered with isolates from South Pacific, and South East Asia, in phylogenetic analysis of the rDNA internal transcribed spacer (ITS). Given that only three isolates from Big Island and Kauai

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shared higher sequence identities to isolates from the China and India. It is possible that *P. colocasiae* in Hawaii are from two origins.

(Keywords: ***Phytophthora colocasiae***, distribution, mating type, metalaxyl, ITS region)

INTRODUCTION

Taro (***Colocasia esculenta***) is a Polynesian staple food, grown on all of the major islands of Hawaii and throughout the Asia-Pacific region. In 2011, the yield of taro in Hawaii was estimated at 4 million pounds [33]. Its hypoallergenic characteristic, high starch content and mild taste make it a valuable crop for export and domestic consumption. Taro leaf blight caused by ***Phytophthora colocasiae***, however, greatly limits production during wet periods. Severe leaf infections followed by 50% to 100% of defoliation strongly hinders photosynthesis, leading to stunted corms and yield reduction. Mycelia of ***P. colocasiae*** penetrate harvested corms and cause heavy post harvest losses. Yield reductions of 15 to 45% are common, and can reach as high as 80% during severe foliar epidemics [22]. Vegetative propagules (shoot cuttings) are infected and reduce establishment of new fields. Sporangia are important to disease dispersal and as survival structures [28], are viable for up to 21 d in naturally-infested soil [6] and approximately 1 mo in taro leaves [7]. Gomez (1925) and Quitugua and Trujillo (1998) reported that sporangia formed on taro leaf blight lesions and survived up to 107 d in soil at 75% moisture [7, 24].

Among ***Phytophthora*** species, genetic variation increases after sexual reproduction, which is associated with the production of progeny of varying virulence [8, 19, 34]. ***Phytophthora colocasiae*** is predominantly heterothallic, and produces sexual spores (oospores) only when both A1 and A2 mating types are present. Hainin Island, China is thought to be within the center of origin, as the ratio of A1 and A2 mating types of ***P. colocasiae*** is 136:102 (approximately 1:1) [37]. In other parts of the world where ***P. colocasiae*** is found, the presence of only a single mating type or the predominance of one mating type is thought to reflect the mating type of the founder isolates. For example, in 1979, 101 isolates of ***P. colocasiae*** from the Big Island of Hawaii were all of the A1 mating type, as were 8 and 5 isolates from Maui and Kauai, respectively [14]. A survey of populations in North India in 1980 also identified only A1 isolates [20]. However, in the resurvey of ***P. colocasiae*** in the Pacific region from 1997 to 2001, 16, 5, 5, 5, and 3 isolates collected in Hawaii, North India, Papua New Guinea, Samoa and Guam, respectively, were all of the A2 mating type [30]. A total of 799 isolates collected from 15 counties in Taiwan also were of the A2 mating type

[1]. In addition, seven homothallic (A1A2 type that formed oospores without crossing) were observed in Chiayi, Taiwan in 2006 to 2007[18].

Metalaxyl is a systemic fungicide used to control various diseases caused by ***Phytophthora*** spp. In Hawaii, it is frequently used to control ***Phytophthora*** and ***Pythium*** on foliage plants such as Aglaonema, Pothos, and Schefflera, on bedding plants such as Catharanthus and impatiens, and on flowers such as African violets, geranium, and poinsettia. It is highly effective, but it is also toxic to aquatic organisms such as green algae [36], and resistance has been recorded in several ***Phytophthora*** spp., including ***P. infestans*** [5], ***P. capsici*** [23], and ***P. cinnamomi*** [11]. In addition, resistant isolates of ***P. infestans*** were reported to have changed from A1 to A2 and from A2 to A1 after exposure to metalaxyl or mefenoxam [4, 8]. No fungicides are currently registered for use in wetland taro production in Hawaii [32]. However, metalaxyl can be used as a pre-plant treatment for soaking taro plants, before planting. Use of metalaxyl and mefenoxam is common in foliage nurseries and run off from these nurseries flow to taro paddies. Thus, extended periods of soaking in metalaxyl or repeated environmental exposure to these pesticides may select for resistance and cause mating type change in ***P. colocasiae***.

Phytophthora colocasiae is known from most taro-producing regions throughout the Pacific and Asia [1, 9, 14, 20, 30, 37]. Raciborski was the first to describe taro leaf blight in Java in 1900 [25] followed by Sawada, in Taiwan in 1911 [26]. The exact route of spread from Java to Taiwan is not known, and different authors provide alternative pathways: Ooka [21] postulated that the disease was spread from Java to Taiwan via the northern route, whereas Trujillo speculated dispersal via a southern route from Java to the Pacific region [29]. The disease was first reported in the Philippines in 1916, and later in Micronesia and Guam [2]. Since 1948, the disease has been found in Papua New Guinea, Australia, the Solomon Islands, and Fiji. It is believed to have been introduced to Hawaii from Taiwan and Japan in 1920 [3, 21]. ***Phytophthora colocasiae*** is consistently and most common associated with ***Colocasia esculenta***. Species of ***Alocasia*** and ***Xanthosoma*** have been reported as hosts but the pathogen has not been found on these plants in Hawaii. Thus for ***Phytophthora colocasiae***, ***C. esculenta*** is the major host.

The objectives of this study were to determine the current distribution of mating types and metalaxyl resistance of ***P. colocasiae*** in Hawaii, and to compare the Hawaii population to representative isolates from other populations in a phylogenetic analysis of the rDNA internal transcribed spacer (ITS) region.

MATERIALS AND METHODS

Isolation of *Phytophthora colocasiae*

Diseased taro leaves were collected from Kauai, Oahu, Molokai, and the Big Island, and sent to the University of Hawaii at Manoa for examination and isolation. Leaves were washed with dilute liquid detergent and rinsed with running tap water to remove surface debris and contaminants. Five pieces (ca. 5 × 10 mm) were cut from each leaf, surface sterilized in 0.6% sodium hypochlorite for 2 min, blotted on a sterile paper towel, and placed on 1.5% water agar. Plates were incubated at 24 ˚C under continuous fluorescent light. Mycelia of *P. colocasiae* were visible after 1 to 3 d. From each diseased leaf, 2 single-hyphal tips were transferred to V8 agar (10% V8 juice, 0.025% $CaCO_3$, 1.8% Bacto agar) (V8 juice, Campbell Soup Co., U.S.A.) and incubated at 24 ˚C under continuous fluorescent light. To induce sporulation, an agar plug from a 5-d-old culture was submerged in 10 ml sterile distilled water, incubated at 24 ˚C under continuous fluorescent light for 16 to 24 h, and then transferred to 16 ˚C for 30 min [18, 37]. A small amount of the zoospore suspension was then spread on 2% V8 agar (2% V8 juice, 0.005% $CaCO_3$, 1.8% Bacto agar) using a sterile loop. After incubation at 24 ˚C under light for 6 h, individual germinated zoospores were transferred to V8 agar plates. These single-zoospore cultures were used for subsequent study. Sporangia were also produced on the agar surface on V8 agar at 24 ˚C in 4-6 d.

Morphology of *P. colocasiae* and colony radius

Isolates grown at 24 ˚C under fluorescent irradiation for 4-6 d were observed under a light microscope at 200 X for sporangial characteristics using water mounts. Oogonia, antheridia and oospores were also observed at 200 X. The linear growth of each isolate was measured by placing a 5-mm-diameter culture disk taken from a culture actively growing on V8 agar and placing it on V8 agar plate. After incubation at 24 ˚C in darkness for 4 d, the growth was measured from the edge of the inoculum disk to the point in the colony where the mycelia had grown the greatest distance from the disk. Three measurements were taken for each colony.

Determination of mating type

The mating type of each isolate was determined by pairing a small piece (3 mm × 3 mm) of culture growing on V8 agar, with a similar piece of culture of A1 (H1687, from papaya) or A2 (H1769, from cacao) testers of *P. palmivora* on a 15-mm V8 agar disk containing 0.5% ground rape seed, in a 24-well culture plate (#5530300, Orange Scientific, Belgium), with each well measuring 15 mm wide × 17 mm deep. Ground rape seeds were prepared by using a mortar and pestle to make a fine powder,

which was added before autoclaving to V8 agar, making V8-rape agar. After incubation at 24 ˚C in darkness for 7 to 10 d, agar blocks were observed under a light microscope. Isolates forming oospores when paired with the A2 tester were designated as A1, while isolates forming oospores when paired with the A1 tester were designated as A2 [16]. In initial tests, isolates that did not produce oospores on V8 agar when paired with A1 or A2 tester, were designated A0 [36]. Several tests were maintained for 1 or 2 mo and re-examined weekly for oospores.

Determination of resistance to metalaxyl

A culture disk was placed on V8 agar amended with 0, 3, 10, 30 or 100 ppm metalaxyl (Ridomil 2EC, USA) and incubated at 24 ˚C in darkness for 4 d. Measurements were made similar to growth rate experiments. Three to ten single-zoospore isolates from each location were tested, and three replicates of each isolate were used.

DNA extraction, sequencing and phylogenetic analysis

A piece (5 mm × 5 mm) of V8 agar culture was placed on a piece (60 mm × 60 mm) of sterile cellophane (#165-0963, Bio-Rad, USA) laid on V8 agar in a 9-cm Petri dish. About 0.02 g of mycelium was collected after 3-5 d at 24 ˚C with light, and placed in a sterile 1.5-ml microcentrifuge tube with 100 µl of TE buffer (10 mM Tris-HCl, 1 mM Na_2-EDTA, pH 8.0) using a scalpel and kept at -20 ˚C for 2 to 4 h. The DNA was extracted twice by heating in a 1000 watt microwave oven set on "high" for 2 min. The mycelium mat was centrifuged at 6000 g for 1 min. Five to 10 µl of the supernatant with DNA was used for polymerase chain reaction (PCR). The ITS sequence was amplified with primer pair of ITS1 and ITS4 [35]. PCR was performed in a 50 µl volume reaction containing 0.5 pmol each of ITS1 and ITS4 primers with buffer system (2X ImmoMix, BIOLINE, USA) recommended by the manufacturer. Cycling conditions for the PCR reaction were: initial denaturation at 95 ˚C for 7 min, 35 cycles of 95 ˚C for 30 s, 55 ˚C for 30 s, 72 ˚C for 1 min and final elongation at 72 ˚C for 6 min. Amplified PCR products were analyzed by electrophoresis in a 1.2% agarose gel and sequenced by an automatic DNA sequencer (ABI PRISM 377, Perkin-Elmer, California) with the BigDye Terminator Cycle Sequencing Kit (Applied Biosystem, Perkin-Elmer, California). To examine phylogenetic relationships, the ITS sequences were aligned by the multiple alignment algorithm using Clustal W method and phylogenetic trees were constructed by the neighbour-joining method in MEGA5 [27]. The percentage of replicate tree in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

RESULTS

Isolation of *Phytophthora colocasiae* from diseased taro leave

Phytophthora blight of taro was found in 20 farms in 12 districts, and a total of 141 isolates of *P. colocasiae* were recovered from symptomatic leaves (Fig. 1, Table 1). In 1997, 18 isolates were recovered from taro in Kahalu'u and Waimanalo, both on the island of Oahu. From 1999 to 2002, 40 and 19 isolates were obtained from Hakalau and Waipio Valley of the Big Island, respectively. In 2011, 28 farms in 17 districts of Oahu, Kauai, Molokai and the Big Island were surveyed. *Phytophthora colocasiae* was not found in taro farms sampled in the districts of Koloa, Kaneohe, Nu'uanu, Pearl City or Poamoho.

Morphology and radial growth rates of *Phytophthora colocasiae isolates*

Numerous semipapillate, caduceus sporangia with short to medium length pedicels, averaging 6.1 μm, were observed on V8 agar in the water but spherical chlamydospores were not observed. Most isolates were heterothallic with amphigynous

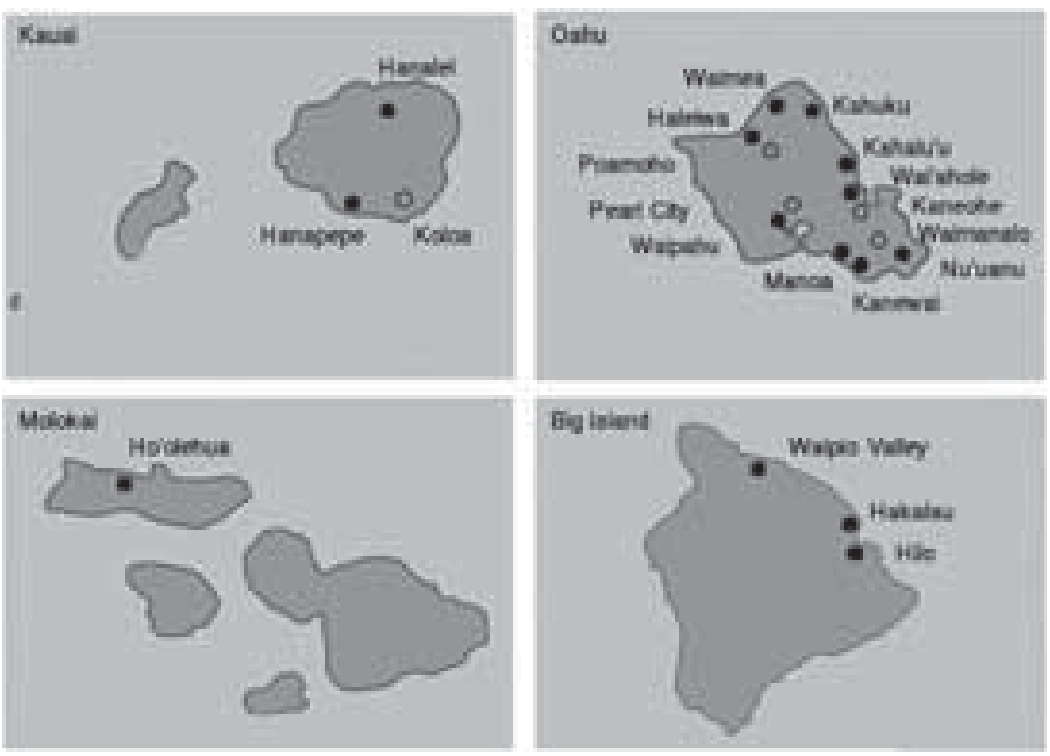

Fig. 1. Distribution of *Phytophthora colocasiae* in taro fields of Hawaii from 1997 to 2011. Black circles indicate the presence of *P. colocasiae*, whereas white circles indicate its absence.

Table 1. Distribution of ***Phytophthora colocasiae*** in taro fields in Hawaiian

Island	District	No. of isolates recovered	Mating type		Year of isolation
			A1	A2	
Kauai	Hanalei	52	0	52	2011
	Koloa	0	--[a]	--	2011
	Hanapepe	5	0	5	2011
Oahu	Kahuku	24	0	24	2011
	Kahalu'u	10	0	10	1997
	Kahalu'u	5	0	5	2011
	Wai'ahole	2	0	2	2011
	Kaneohe	0	--	--	2011
	Waimanalo	8	0	8	1997
	Nu'uanu	0	--	--	2011
	Kanewai	8	0	8	2011
	Manoa	5	0	5	2011
	Waipuha	2	0	2	2011
	Pearl City	0	--	--	2011
	Poamoho	0	--	--	2011
	Haleiwa	10	0	10	2011
	Waimea	11	0	11	2011
Molokai	Ho'olehua	14	0	14	2011
Big Island	Waipio Valley	19	0	19	2002
	Hakalau	1	0	1	1999
	Hakalau	39	0	39	2002
	Hilo	3	0	3	2011

[a] "--", none detected.

antheridium, spherical oogonium, aplerotic oospores. Radial growth of the 141 isolates collected in 2011 was measured on V8 agar, with the average linear growth of the isolates ranging from 10 to 20 mm. Approximately 79% of the isolates grew from 13 to 17 mm in 4 d (Fig. 2). Two types of colony morphologies were observed. Type I showed sparse aerial mycelia, with uniform colony growth in concentric rings, and a smooth colony margin (Fig. 4A). Type II showed dense cottony aerial mycelia

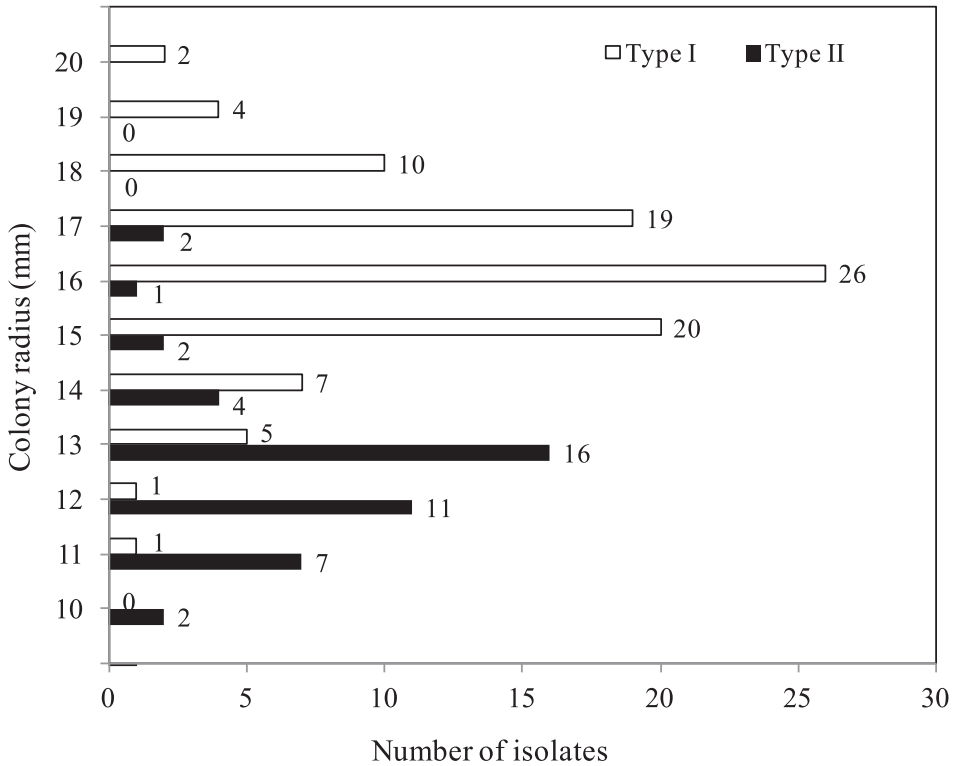

Fig. 2. Radial growth in mm of ***P. colocasiae*** isolates incubated on V8 agar at 24 ˚C for 4 d with type I and type II colony morphology and the number of isolates for each type.

with an irregular colony margin. Among the isolates obtained from 20 farms in Hawaii, isolates from 8 farms belonged to type I, 6 farms belonged to type II, and isolates from 6 farms consisted of both types I and II.

Determination of mating type

In our initial attempts to determine mating type of a subset of 48 isolates, using V8 agar to pair our isolates with A1 and A2 tester isolates, 15 isolates were identified as A2 and 33 were A0 (i.e., neither A1, nor A2). However, when these isolates were retested on V8 agar supplemented with 0.5% ground rape seed (V8-rape agar) all 48 isolates behaved as the A2 mating type. As such, V8-rape agar was selected as the culture medium for determination of mating type. Isolates collected from Oahu and the Big Island from 1997 to 2002 were found to be A2 type (Table 1), based on the formation of oospores when paired with a tester isolate of the A1 mating type, but not the A2 mating type, on V8-rape agar. Also, isolates collected in 2011, from Kauai, Oahu, Molokai, and the Big Island, were found to be A2 type.

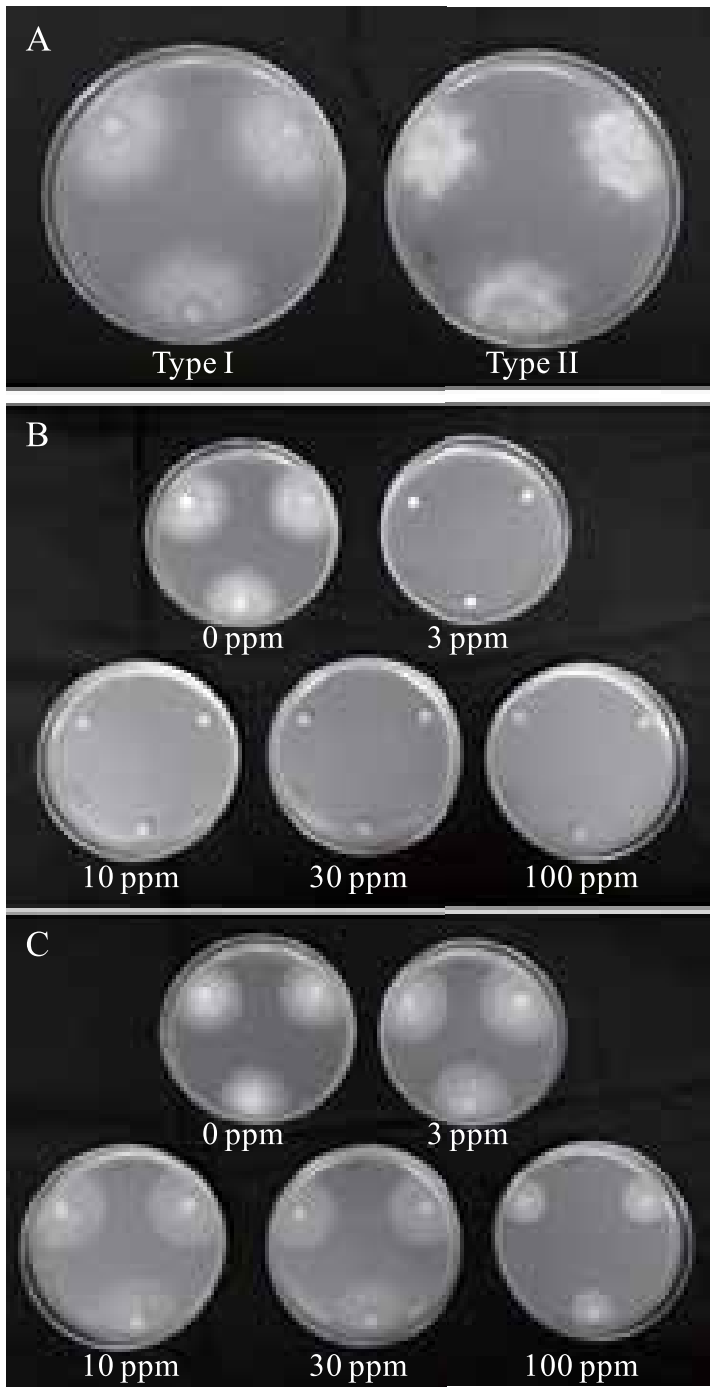

Fig. 4. Colony morphology of ***Phytophthora colocasiae*** isolates. A, exhibiting type I and type II morphologies for isolates GS24 and GS12, respectively. B, colony growth on agar of metalaxyl sensitive isolate GS20 and C, resistant isolate GS36 at 0, 3, 10, 30 and 100 ppm metalaxyl.

Determination of metalaxyl resistance

Of the 141 isolates collected in 2011, 17 isolates from Oahu were resistant, based on their ability to grow on V8 agar containing 30 ppm metalaxyl at a growth rate similar to that of V8 agar without metalaxyl. The remaining isolates were metalaxyl-sensitive, and were incapable of growth even at 3 ppm metalaxyl. For example, the sensitive isolates GS20 and GS55 were completely inhibited at all the concentrations tested (Fig. 3, 4B). In contrast, isolates GS24, GS36 and GS62 grew at similar rates on V8 agar with 0 or 30 ppm metalaxyl (Fig. 3, 4C). The colony morphology of metalaxyl-resistant isolates all belonged to type I. The aerial mycelium was reduced and became sparse with increasing concentrations of metalaxyl. Even at 100 ppm metalaxyl, GS24, GS36, GS62 were still capable of growth, albeit at a slower growth rate, at about 50% of that on V8 agar without metalaxyl.

Phylogenetic analysis

In phylogenetic analysis of ITS, 21 isolates collected from Oahu, Kauai, Molokai and Big Island of Hawaii in 2011 were clustered with known sequences from South Pacific and South East Asia

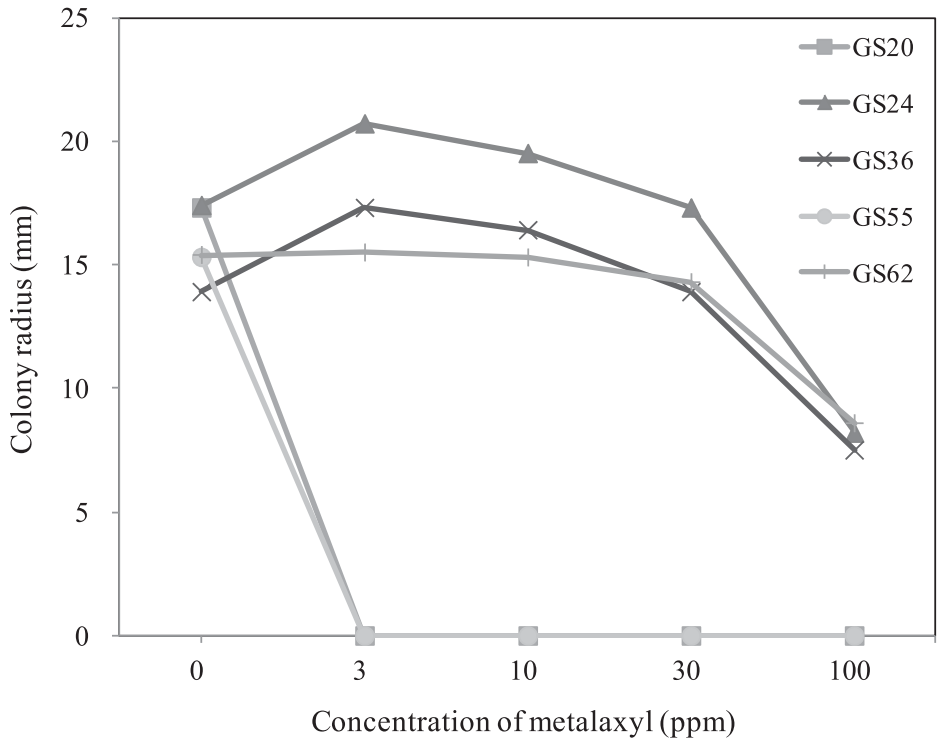

Fig. 3. Average radial growth in mm of *P. colocasiae* at 24 ˚C for metalaxyl sensitive isolates GS20 and GS55 compared to resistant isolates GS24, GS36 and GS62 on 0, 3, 10, 30 and 100 ppm metalaxyl in 10% V8 agar after 4 d.

(Table 2, Fig. 5). Eighteen of 21 isolates collected from were clustered into the same group with *P. colocasiae* from Taiwan (GU111605), Japan (AB367386), Indonesia (GU258989), American Samoa (GU258960), Malaysia (GU983631), Papua New Guinea (AJ516028), Thailand (AJ516031), Viet Nam (AJ516032), the Philippines (GU259002), and Hawaii (GU258998). The sequence identities were from 99.6 to 100.0%. However, the isolates BI1 (JN661138), KH31 (JN661144) and KH42 (JN661145) that clustered with high nucleotide sequence identities to *P. colocasiae* in India (JN315692, JX134654, GU259005), China (HQ848082, AF363009), and Nigeria (HQ602756). The identities of the ITS sequences ranged from 96.4 to100.0%.

DISCUSSION

Genetic variation has been observed to increase after sexual reproduction, leading to the production of variable virulence in progenies in *P. infestans* and *P. ramorum* [8, 19, 34]. *Phytophthora colocasiae* is generally considered heterothallic, although, seven homothallic isolates were found in Taiwan [18]. The homothallic isolates produced abundant oospores in taro tissues, suggesting that oospores may serve as survival structures, and as a source of genetic variation in nature [18]. In 1979, Ko reported that all of the 114 isolates collected from the Big Island, Maui, and Kauai were A1 mating type [14]. However, in this study, all *P. colocasiae* isolates obtained from Oahu, Kauai, Molokai, and the Big Island were A2 mating type. This result was consistent with previous reports that 16 isolates obtained from Hawaii were all A2 type [30]. No A1 mating type isolates could be found, indicating that only A2 mating type is currently established in Hawaii. The reason for the complete change of mating type of *P. colocasiae* from A1 to A2 in Hawaii is unknown. Previously, *P. infestans* on the island of Hawaii also changed from all A1 to all A2 within 10 years [10]. The conversion of mating type in *P. infestans* and *P. parasitica* on medium containing metalaxyl has been reported [4]. Some metalaxyl resistant isolates of *P. infestans* also change mating types after exposure to metalaxyl or mefenoxam [8] as mentioned previously.

Among the Hawaii isolates obtained in this study, two types of colony morphologies were observed, but no chlamydospores formed on V8 agar. In initial studies, approximately one third of the *P. colocasiae* isolates did not produce oospores on V8 agar when paired with A1 or A2 tester, and were designated A0. However, these A0 isolates did form oospores when paired with the A1 tester isolate, but not A2 tester, on V8 agar amended with ground rape seed. This is consistent with previous observations, suggesting that some chemical

Table 2. Isolates recovered from taro fields and known isolates from previous studies of ***Phytophthora colocasiae***, for confirmation of identity of taro isolates in phylogenetic analysis of the nuclear rDNA internal transcribed spacer region (ITS)

GenBank accession no.	Isolate	Size of ITS region (bp)	Origin	Submitor
AB367386	P6317	779	Japan	Uddin et al. (2007)
AF266786	IMI368918	777	Malaysia	Cooke et al. (2000)
AF363009	NJYu1	779	China	Shen et al. (2001)
AJ516028	PNG4	779	Papua New Guinea	Herail et al. (2002)
AJ516029	PH12	779	Philippines	Herail et al. (2002)
AJ516031	TH37	779	Thailand	Herail et al. (2002)
AJ516032	VT2	779	Viet Nam	Herail et al. (2002)
AY251655	20216	779	Taiwan	Huang and Liou (2003)
FJ801356	WPC10273A595	780	American Samoa	Coffey et al. (2009)
GQ202148	98-111	782	India	Mishra et al. (2009)
GU111605	TARI 98115	779	Taiwan	Ann et al. (2009)
GU258960	WPC10701A1970	779	American Samoa	Coffey et al. (2009)
GU258991	WPC6397A1974	779	Indonesia	Coffey et al. (2009)
GU258997	WPC 7172A1946	779	Hawaii	Coffey et al. (2009)
GU258998	WPC 7174A1917	777	Hawaii	Coffey et al. (2009)
GU258999	WPC 7175A1926	777	Hawaii	Coffey et al. (2009)
GU259002	WPC 7538A1863	780	Philippines	Coffey et al. (2009)
GU259003	WPC 7627A1944	783	Taiwan	Coffey et al. (2009)
GU259005	WPC 8170A1872	779	India	Coffey et al. (2009)
GU983631	WPC 6947D66	779	Malaysia	Coffey et al. (2009)
HQ602756	Pc01	782	Nigeria	Bandyopadhyay et al. (2010)
JN661138	BI1	779	Big Island (Hilo)	This study
JN661139	BI3	779	Big Island (Hilo)	This study
JN661142	GS36	779	Oahu (Kahuku)	This study
JN661143	KH6	779	Kauai (Hanalei)	This study
JN661144	KH31	779	Kauai (Hanapepe)	This study
JN661145	KH42	777	Kauai (Hanalei)	This study
JN661146	MK76	779	Molokai (Ho'olehua)	This study
JX134654	CTCRI-PC-112-12	780	India	Vishnu et al. (2011)

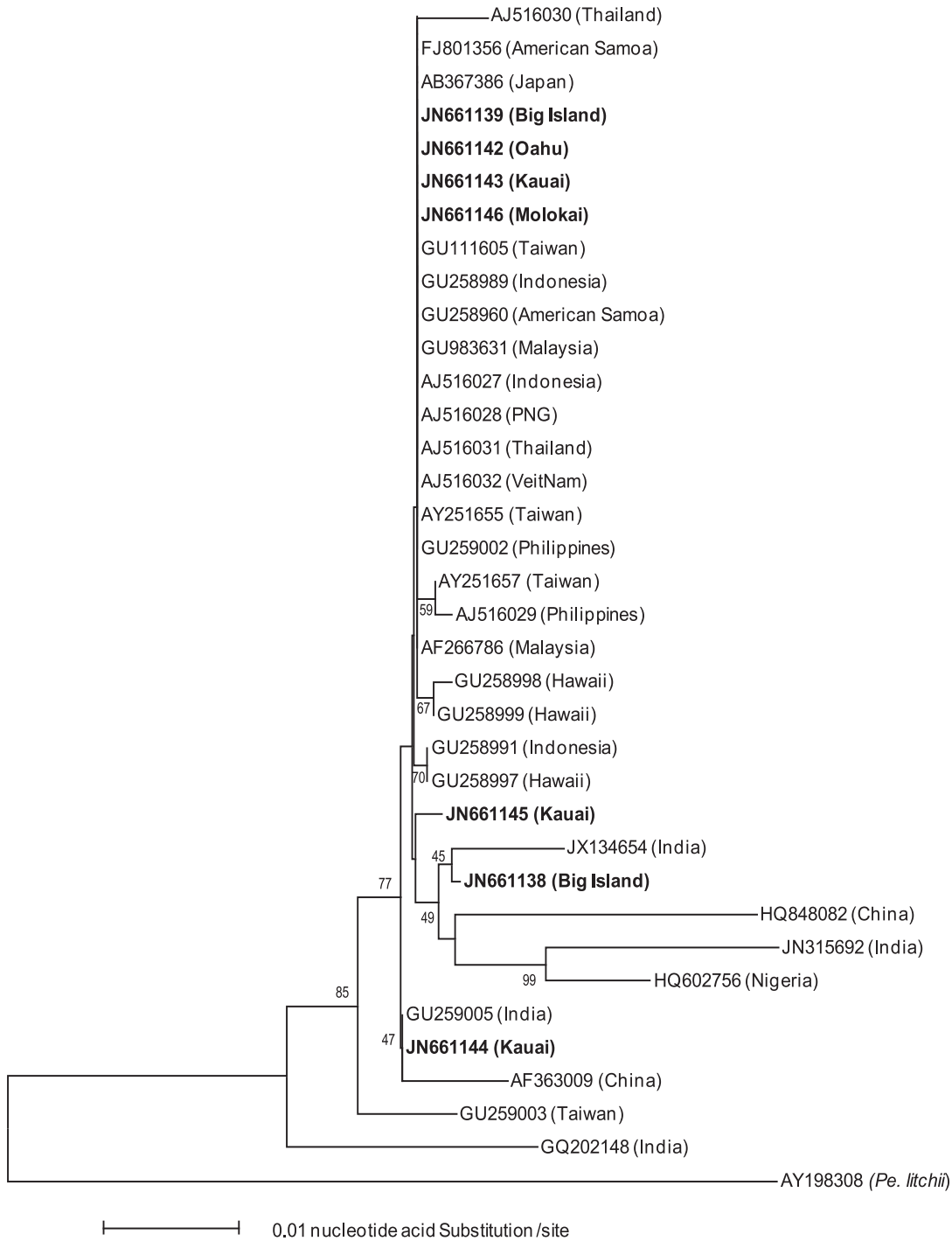

Fig. 5. Phylogenetic trees were based on the nuclear rDNA internal transcribed spacer region (ITS) using neighbor-joining method. ***Phytophthora colocasiae*** isolates that we recovered from taro fields in Hawaii are in bold-face type.

nutrient or compound from rape seed is stimulatory to oospore formation [31]. Additionally, *P. colocasiae* isolates from Taiwan that formed abnormal oospores on V8 agar produced normal oospores on V8 agar amended with ground rape seed (M. J. Lin unpublished data). Previous reports showed that lecithin, cephalin, and fatty acids also stimulate oospore formation in *P. parasitica* and *P. cactorum* [12, 15, 17]. It is possible that A0 mating type isolates found in Hainan Island and Papua New Guinea and reported to be the A0 mating type [30, 37] are actually A1 or A2, but were not categorized as such because matings were not conducted on the proper medium.

Among the isolates of *P. colocasiae* collected in Hawaii in 2011, metalaxyl-resistant isolates were found only on Oahu. Although metalaxyl is not registered for use in taro paddies [32], the water that originates in the mountains flows into the urban/agricultural areas, e.g., Manoa Valley above the University. The water carries metalaxyl from nurseries and ornamental farms, where use of metalaxyl is very common. Thus, paddy plots that use this water downstream will be exposed to metalaxyl in the water and sediments, and the chemical may accumulate and remain in the mud of the paddy for months. Metalaxyl-resistant isolates of *P. infestans* produced more oospores on medium amended with metalaxyl than metalaxyl-sensitive isolates [8]. The presence of fungicide-induced oospores in the field indicates sexual reproduction is occurring, which leads to increased genetic variability in the pathogen population [8]. Whether this is the case among the metalaxyl-resistant isolates of *P. colocasiae* on Oahu needs to be investigated.

Based on the phylogenetic analysis, most isolates of *P. colocasiae* obtained from Hawaii were similar to isolates from Taiwan, Indonesia, American Samoa, Malaysia, Papua New Guinea, Thailand, Viet Nam, the Philippines. One isolate from Big Island and two isolates from Kauai were closer to isolates from China and India. The theory that *P. colocasiae* moved to Hawaii through Taiwan and Japan in 1920 [21]. However, historical records indicate that *P. colocasiae* was present in Hawaii as early as 1918 [3], and it was first reported in Japan in 1967 [13]. Our studies suggests that *P. colocasiae* in Hawaii were probably from two origins.

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REFERENCES

1. 1. Ann, P. J., and Ko, W. H. 1986. Mating-type distribution of ***Phytophthora colocasiae*** in Taiwan. Mycopathologia 93: 193-194.
2. Brunt, J., Hunter, D., and Delp, C. 2001. A bibliography of taro leaf blight. Secretariat of the Pacific Community publishing, Noumea, New Caledonia. 96 pp.
3. Carpenter, C. W. 1920. Report of the plant pathologist, pp. 49-54. ***In***: Hawaii Agricultural Experiment Station [eds.], The report of Hawaii Agricultural Experiment Station Report for 1919, Hawaii, USA. 73 pp.
4. Chang, T. T., and Ko, W. H. 1990. Effect of metalaxyl on mating type of ***Phytophthora infestans*** and ***P. parasitica***. Ann. Phytopthol. Soc. Jpn. 56: 194-198.
5. Cohen, Y., and Reuveni, M. 1983. Occurrence of metalaxyl-resistant isolates of ***Phytophthora infestans*** in potato fields in Israel. Phytopathology 73: 925-927.
6. Gollifer, D. E., Jackson, G. V. H., and Newhook, F. J. 1980. Survival of inoculum of the leaf blight fungus ***Phytophthora colocasiae*** infecting taro, Colocasia esculenta in the Solomon Islands. Ann. Appl. Biol. 94: 379-390.
7. Gomez, E. T. 1925. Leaf blight of Gabi (***Phytophthora colocasiae*** Rac.) in Philippines. Philipp. Agric. 14: 429-440.
8. Groves, C. T., and Ristaino, J. B. 2000. Commercial fungicide formulations induce in vitro oospore formation and phenotypic change in mating type in ***Phytophthora infestans***. Phytopathology 90: 1201-1208.
9. Ho, H. H., Hu, Y. N., Zhuang, W. Y., and Liang, Z. R. 1983. Mating types of heterothallic species of ***Phytophthora*** in China. Acta Mycol. Sin. 2: 187-191.
10. Ho, W. H., and Ko, W. H. 1999. Temporal distribution of mating types and cultural variation of ***Phytophthora infestans*** at the Volcano district on the island of Hawaii. Ann. Phytopathol. Soc. Jpn. 65: 286-292.
11. Hu, J., Hong, C., Stromberg, E. L., and Moorman, G. W. 2010. Mefenoxam sensitivity in ***Phytophthora cinnamomi*** isolates. Plant Dis. 94: 39-44.
12. Jee, H. J., Tang, C. S., and Ko, W. H. 1997. Stimulation of sexual reproduction in ***Phytophthora cactorum*** by phospholipids is not due to sterol contamination Microbiology 143: 1631-1638.
13. Katsure, K. 1971. Phytophthora Diseases of Plants. Seibundo-Shinkosha, Tokyo. 128 pp.
14. Ko, W. H. 1979. Mating-type distribution of ***Phytophthora***

colocasiae on the island of Hawaii. Mycologia 71: 434-437.

15. Ko, W. H. 1985. Stimulation of sexual reproduction in ***Phytophthora cactorum*** by phospholipids. J. Gen. Microbiol. 131: 2591-2594.
16. Ko, W. H. 1994. An alternative possible origin of the A2 mating type of ***Phytophthora infestans*** outside Mexico. Phytopathology 84: 1224-1227.
17. Ko, W. H., and Ho, W. C. 1983. Reassessment of apparent sterol requirement for sexual reproduction in ***Phytophthora***. Ann. Phytopathol. Soc. Jpn. 49: 316-321.
18. Lin, M. J., and Ko, W. H. 2008. Occurrence of isolates of ***Phytophthora colocasaie*** in Taiwan with homothallic behavior and its significance. Mycologia 100: 727-734.
19. Mayton, H., Smart, C. D., Moravec, B. C., Mizubuti, E. S. G., Muldoon, A. E., and Fry, W. E. 2000. Oospore survival and pathogenicity of single oospore recombinant progeny from a cross involving US-17 and US-8 genotypes of ***Phytophthora infestans***. Plant Dis. 84: 1190-1196.
20. Narula, K. L., and Mehrotra, R. S. 1980. Occurrence of A1 mating type of ***Phytophthora colocasiae***. Indian Phytopathol. 33: 603-604.
21. Ooka, J. J. 1980. Taro diseases. Hawaii Institute of Tropical Agriculture and Human Resources, Honolulu, Hawaii, Research Extension Series 44: 51-59.
22. Paiki, F. A. 1996. Symptoms of taro leaf blight disease (***Phytophthora colocasiae***) and relationship with yield components in Biak, Irian Yaya. Sci. New Guinea 21: 153-157.
23. Parra, G., and Ristaino, J. B. 2001. Resistance to mefenoxam and metalaxyl among field isolates of ***Phytophthora capsici*** causing Phytophthora blight of bell pepper. Plant Dis. 85: 1069-1075.
24. Quitugua, R. J., and Trujillo, E. E. 1998. Survival of ***Phytophthora colocasiae*** in field soil at various temperatures and water matric potentials. Plant Dis. 82: 203-207.
25. Raciborski, M. 1900. Parasitic algae and fungi. Java Batavia Bull. 19: 189.
26. Sawada, K. 1911. ***Phytophthora*** disease of Taro. Spec. Bull. Agr. Exp. Sta. Gov. Formosan 2: 75-84.
27. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol. Biol. Evol. 28: 2731-2739.
28. Trujillo, E. E. 1965. The effects of humidity and temperature on Phytophthora blight of taro. Phytopathology 55: 183-188.
29. Trujillo, E. E. 1967. Diseases of the genus ***Colocasia*** in Pacific area and their control. Proc. Int. Symposium on

Tropical Root Crops 2: 13-19.

30. Tyson, J. L., and Fullerton, R. A. 2007. Mating types of ***Phytophthora colocasiae*** from the Pacific region, India and South-east Asia. Austral. Plant Dis. Notes 2: 111-112.
31. Uchida, J.Y. and Aragaki, M. 1980. Chemical stimulation of oospore formation in ***Phytophthora capsici***. Mycologia 72: 1103-1108.
32. Uchida, J. Y., Sliva, J. A., and Kadooka, C. Y. 2002. Improvements in taro culture and reduction in disease levels. Cooperative Extension Service, CTAHR, University of Hawaii PD 22: 1-4.
33. United States Department of Agriculture: National Agriculture Statistics Service. 2011. Statistics of Hawaii Agriculture. http://www.nass.usda.gov/Statistics_by_State/Hawaii/Publications/Annual_Statistical_Bulletin/index.asp.
34. Vercauteren, A., Boutet, X., D' hondt, L., Bockstaele, E. V., Maes, M., Leus, L., Chandelier, A., and Heungens, K. 2011. Aberrant genome size and instability of ***Phytophthora ramorum*** oospore progenies. Fungal Genet. Biol. 48: 537-543.
35. White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. pp. 315-322. In: M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR protocols: a guide to methods and applications. Academic Press, San Diego, CA. 482 pp.
36. Yao, K., Zhu, L., Duan, Z., Chen, Z., Li, Y., and Zhu, X. 2009. Comparison of ***r***-metalaxyl and ***rac***-metalaxyl in acute, chronic, and sublethal effect on aquatic organisms: ***Daphnia magna***, ***Scenedesmus quadricanda***, and ***Danio rerio***. Environ. Toxicol. 24: 148-156.
37. Zhang, K. M., Zheng, F. C., Li, Y. D., Ann, P. J., and Ko, W. H. 1994. Isolates of ***Phytophthora colocasiae*** from Hainan Island in China: evidence suggesting an Asian origin of this species. Mycologia 86: 108-112.

摘 要

林玫珠[1*]、陳錦桐[2]、Uchida J. Y.[3]、Kadooka C. Y.[3]。2014。芋頭疫病菌的交配型分佈、抗藥性及親緣關係之研究。植保會刊 56(1)：25-42。([1]亞洲大學健康產業管理學系，台中市霧峰區；[2]農業委員會農業試驗所植物病理組，台中市霧峰區；[3]夏威夷大學植物與環境保護科學系，美國夏威夷)

從 1997 到 2011 年，在夏威夷的歐胡島、可愛島、莫洛凱島及大島蒐集罹患芋頭疫病的葉片組織，總共分離到 218 個芋頭疫病菌菌株，其交配型經測試皆為 A2 型。將 2011 年由四個島嶼十二區共二十個田區所蒐集的 141 菌株進行對殺菌劑滅達樂的抗性測試，由可愛島、莫洛凱島及大島蒐集的菌株對滅達樂皆無抗藥性發生，然而，在歐胡島上蒐集的菌株約有百分之二十五對滅達樂具有抗藥性；其中 112 個菌株在 V8 培養基上培養四天，平均生長速度介於 13 到 17 公釐，依其生長於V8培養基之菌落形態可分為二群，一群僅有稀疏的氣生菌絲，另一群菌絲生長較密，具有棉花狀氣生菌絲；所分離的芋頭疫病菌菌株並無產生厚膜孢子。以轉錄區間核糖核酸(ITS)進行親緣分析，有 18 個菌系的轉錄區間核糖核酸序列與南太平洋及東南亞國家的菌株相近；另外一個由大島及二個由可愛島分離的菌株則與中國、印度的菌株具有高度的相同性，因此推論夏威夷的芋頭疫病菌菌株可能源自於二個不同地區。

(關鍵詞：芋頭疫病菌、分佈、交配型、滅達樂、轉錄區間核糖核酸)

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